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Tetramethyltetrazine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The kinetics of oxidation of methyl-hydrazine (MMH) by dissolved oxygen in water has been studied as a function of cupric-ion concentration and pH. The results indicate that the material is not expected to persist in non-acidic waters beyond a period of one or two days. A major oxidation product from both MMH and UDMH is formaldehyde.

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The oxidation of UDMH in UDMH/water mixtures has been studied as a function of the water content of the solution. The formation of dimethylnitrosamine is low at very high UDMH concentrations (80-100% UDMH by volume), optimum in the 50-80% range and lower in more dilute solutions. Extrapolation of these results to environmental conditions reveal that nitrosamine formation is not expected to be significant.

The action of naturally occurring microbial communities on aqueous UDMH solutions has also been investigated. Spectrophotometric analysis indicates that little or no difference in rate or product exists between the test solutions and their sterile controls.

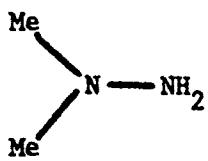
Finally, the uptake of UDMH and/or its oxidation products by aquatic organisms were determined. Uptake by fish was minimal, whereas a slightly higher level was found in Daphnia (biomagnification factor:19-20). An attempt to measure the bioconcentration of tetramethyltetrazene (one of the oxidation products of UDMH) was made, but volatilization of the material from the solution seriously complicated measurement. Nevertheless, the biomagnification factor appears to be of the order of ten.

TABLE OF CONTENTS

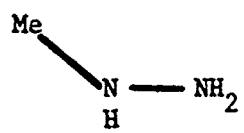
		Page
A.	Introduction	1
B.	Results and Discussion	2
	1. Kinetics and Oxidation of Methylhydrazine	2
	2. Products of Oxidation of MMH	7
	3. Products of Oxidation of UDMN	8
	(i) High UDMH Concentrations	8
	(ii) Low UDMH Concentrations	14
	4. Microbial Degradation of UDMH	15
	5. Bioconcentration of UDMH in Aquatic Organisms	21
	(i) Fish	21
	(ii) Daphnia	28
	6. Bioconcentration of Tetrazene in Fish	30
C.	Summary	31
D.	Experimental Procedures	33
E.	Literature Cited	35
F.	Publications Resulting from the Research	36
G.	Professional Persons Associated with the Project	36
H.	Interactions (Coupling Activities	36

A. Introduction

In the previous phase of the work (1) we established that the oxidation of 1,1-dimethylhydrazine (UDMH) (1) by dissolved oxygen in water was facile, particularly in the presence of transition metal catalysts such as cupric ion. The reaction was pH dependent, the rate being retarded in acidic solutions owing to protonation of the hydrazine. The oxidation led to a complex mixture of products, some of which were identified, whereas others were partially characterized. This report includes data on the oxidation kinetics of methylhydrazine (MMH) (2), identification of the products of oxidation of UDMH, definition of the conditions which control conversion of UDMH to 1,1-dimethylnitrosamine (NDMA) (3), the microbial degradation of UDMH, and the uptake of UDMH and tetramethyltetrazene (4), (one of the oxidation products of UDMH) by fish.



1
(UDMH)



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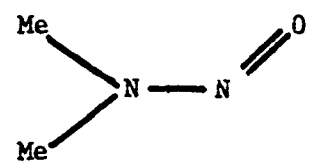
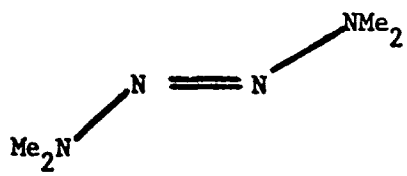
(MMH)



3
(NDMA)



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B. Results and Discussion

1. Kinetics and Oxidation of Methyl-hydrazine

The kinetic procedure used was identical to that described for UDMH in the previous report (1). A solution of the appropriate buffer containing dissolved catalyst was equilibriated to 30.0 $\pm$ 0.1° for at least 10 minutes, and MMH (as the hydrochloride) weighed into the tip of a disposable pipet was introduced into the rapidly stirred solution. The consumption of oxygen was measured by a dissolved oxygen monitor (Yellow Springs International) and the expected pseudo zero-order rate constants (since the concentration of MMH greatly exceeded that of oxygen) were obtained from slopes of the oxygen-time traces, which were linear except for some slight curvature towards the end of the run. The results are tabulated in Table 1, and first-order rate constants derived from plots of $-d[O_2]/dt$ vs. [MMH] are summarized in Table 2. The dependence of the rates on cupric ion concentration at the two acidities studied is illustrated in Figures 1 and 2.

The shapes of the rate profiles are very similar to that observed for UDMH (1), and it is evident that the reaction contains a copper ion catalyzed as well as an uncatalyzed component. Hence, the observed kinetics may be expressed by the equation $k_{obs} = k_1 + k_2\,[Cu^{++}]$ at a given pH, and the curves in Figures 1 and 2 are constructed from the equations $k_{obs} = 6.68 \times 10^{-4}\ min^{-1} \times 1.20 \times 10^{3}\ M^{-1}\ min^{-1}\ [Cu^{++}]$, for the pH = 6.98 runs and $k_{obs} = 6.>4 \times 10^{-3}\ min^{-1} \times 1.81 \times 10^{3}\ M^{-1}\ [Cu^{++}]$ for the pH = 9.16 runs, respectively. Extrapolation of the curve in Figure 2 to very low copper ion concentrations yields a rate constant of approximately $7 \times 10^{-3}\ min^{-1}$. This value compares favorably with the experimentally determined value of $5.64 \times 10^{-3}\ min^{-1}$ (Table 1).

Table 1

Variation of the rate of oxidation of MBH.HCl with $[Cu^{++}]$ and pH

[MBH.HCl] x 10^3M	$[Cu^{++}]$ x 10^7M	pH	k($M.min^{-1}$) x 10^5
6.58	1.00	6.98	2.21
10.2	"	"	1.81
12.6	"	"	2.84
21.0	"	"	3.53
26.3	"	"	3.28
10.3	5.00	"	2.55
10.6	"	"	2.60
16.3	"	"	3.19
31.5	"	"	5.44
3.88	10.00	"	2.45
10.5	"	"	3.58
12.0	"	"	3.92
14.3	"	"	4.17
2.70	60.0	"	3.82
6.50	"	"	6.37
6.87	"	"	8.33
11.6	"	"	11.0
4.34	100.0	"	9.31
4.93	"	"	10.3
11.3	"	"	18.1
2.79	0	9.16	1.23
4.70	"	"	4.29
5.54	"	"	3.43
15.0	"	"	7.11
17.4	"	"	11.5
5.87	5.0	"	2.30
7.83	"	"	3.35
8.61	"	"	3.19
10.2	"	"	3.19
14.8	"	"	3.76
17.6	"	"	5.02
4.39	10.0	"	2.70
7.31	"	"	3.31
9.30	"	"	4.17
13.7	"	"	4.78
20.2	"	"	5.88
30.0	"	"	7.60
4.20	50.0	"	7.84
5.74	"	"	9.80
6.60	"	"	12.7
9.30	"	"	14.7
11.1	"	"	21.3
3.75	100.0	"	13.2
4.39	"	"	16.4
6.99	"	"	26.0
7.29	"	"	26.4
1.82	800.0	"	11.8
2.39	"	"	26.7
3.48	"	"	41.2
3.75	"	"	40.0
4.29	"	"	44.1
4.52	"	"	61.7
9.28	"	"	82.3
9.77	"	"	72.5

Table 2

Summary of the kinetics of oxidation of MMH.HCl

pH	$[Cu^{++}] \times 10^{7}$ M	$k(min^{-1}) \times 10^{4}$	Intercept$(min^{-1}) \times 10^{6}$	CC
6.98	1.00	7.43	15.9	0.84
"	5.00	13.7	11.0	0.998
"	10.00	16.9	18.1	0.997
"	60.0	81.1	17.7	0.97
"	100.0	125.	40.2	0.9996
9.16	0	56.4	- 3.91	0.94
"	5.00	18.5	14.7	0.93
"	10.0	18.7	21.0	0.993
"	50.0	181	- 1.11	0.97
"	100.0	373	- 4.04	0.998
"	800	1500	-131	0.951

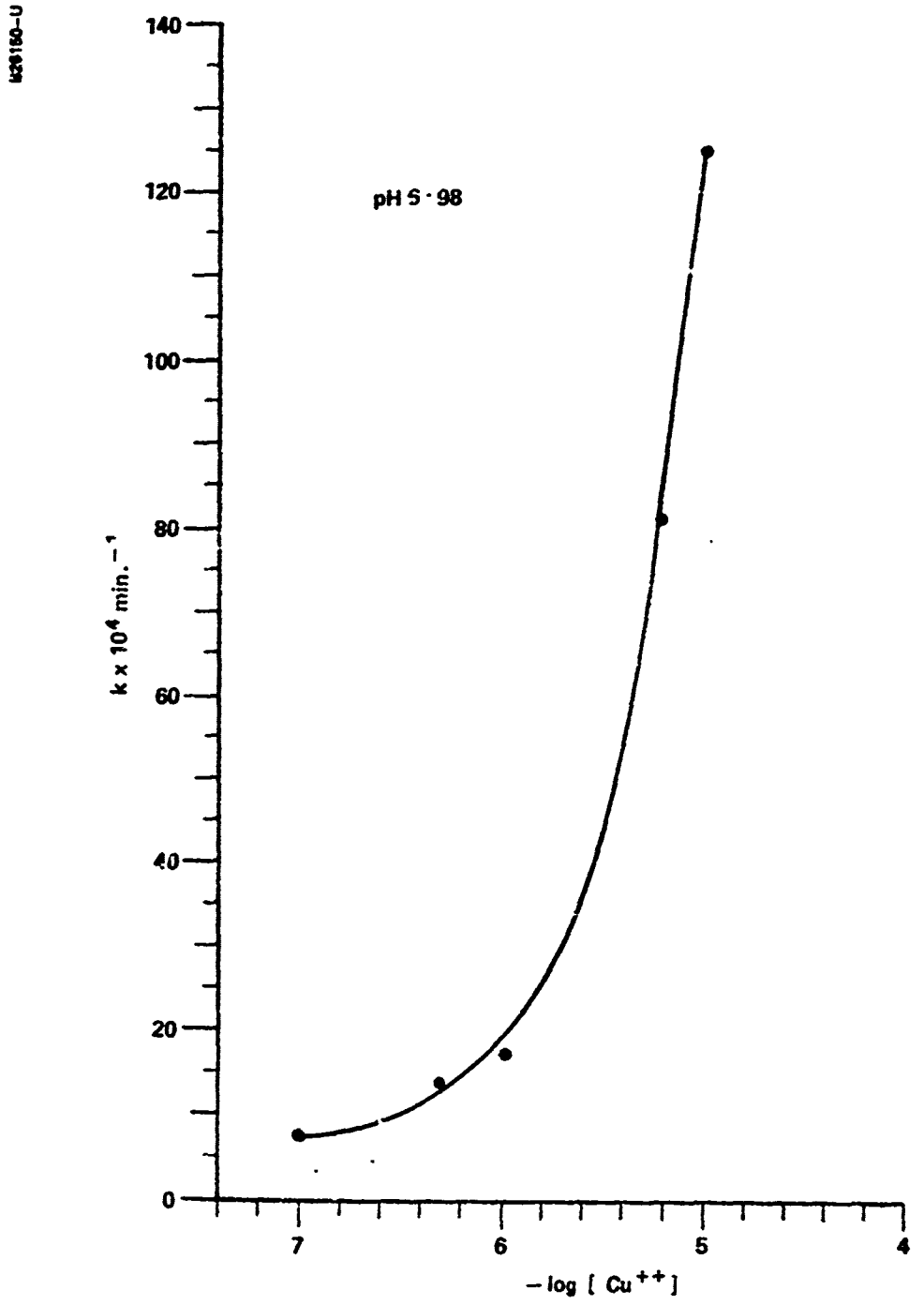


Fig. 1. Variation of the rate of oxidation of MMH with $[Cu^{++}]$ at pH 6.98

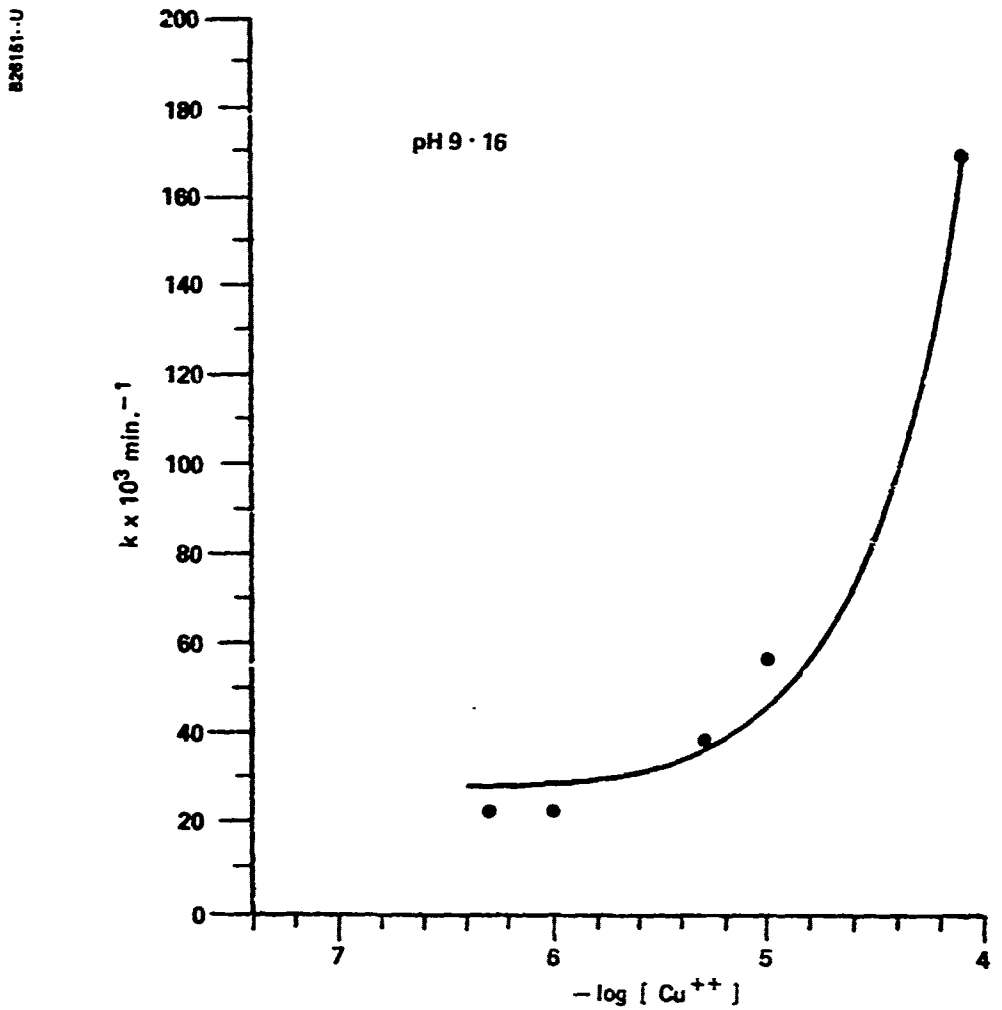


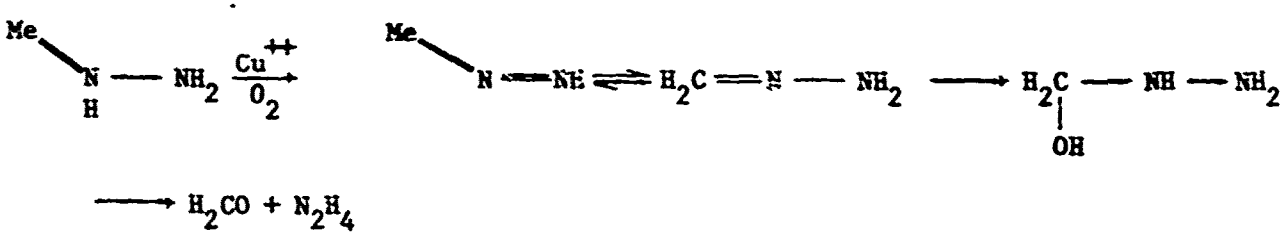
Fig. 2. Variation of the rate of oxidation of MMH with [Cu^{++}] at pH 9.16.

It appears, therefore, that the rate of oxidation of MMH is within an order of magnitude of that of UDMH. The mechanism of the two reactions appear to be similar, in that both processes proceed through the hydrazinyl radical cation. The actual environmental persistence of these compounds will depend largely upon factors like temperature, catalyst concentration, etc., but it is unlikely that these materials will be present for longer than one to two days in non-acidic waters.

2. Products of Oxidation of MMH

Although the oxidation of hydrazine and its dimethyl derivative has been studied to some extent (2), little is known about the corresponding process for MMH. In our initial experiments we reacted solutions of MMH with oxygen in the presence of catalytic amounts of Cu^{++}, and analyzed the reaction mixture by UV spectrophotometry and by HPLC/UV. No UV absorbing materials were found to be present. However, treatment of the solution with chromotropic acid revealed the presence of formaldehyde. A number of experiments were initiated where the formaldehyde concentration was monitored with time. The results presented in Table 3 clearly indicate that the extent of conversion to formaldehyde is not greatly dependent on MMH or Cu^{++} concentration, and is of the order of 30%.

It is currently believed that the oxidation of hydrazine proceeds via diimide intermediates (2). It is therefore likely that similar intermediates are likely to be formed during the oxidation of MMH and the formation of formaldehyde may be rationalized through the following scheme:



3. Products of Oxidation of UDMH

(i) High UDMH Concentration

In our previous studies we noted that the oxidation of UDMH in water did not lead to NDMA. These results contrast with previously reported autoxidation studies (3) in that NDMA is formed in the latter process but not in the former. In order to rationalize these divergent observations, we measured the formation of NDMA as a function of the initial UDMH/water ration under conditions of limiting oxygen. In the first set of experiments, one ml. mixtures of UDMH and water with UDMH/water ratios ranging from 100:0 to 10:90 (by volume) were introduced into a series of flame dried tubes (head space - 25 mls.) which were then sealed under air and allowed to stand in the dark at ambient temperature. The solutions were analyzed after 5, 13 and 14 days for NDMA[1] by HPLC. The NDMA assays were accompanied by fairly large variations (± 30%), and these probably arise from the hygroscopic nature of UDMH, since the extent of NDMA formation is dependent upon the concentration of UDMH (vide infra). The nitrosamine concentration did not vary substantially between the 5, 13 and 14 day samples, and the averaged data are presented in Table 3 and in Fig. 3. Interestingly, the color of the final solution varied with the initial UDMH concentration. The 90 and 100% UDMH solutions remained clear, the 10-30% samples were light yellow and the remaining solutions were brownish yellow.

The results clearly reveal that NDMA formation increases with increasing UDMH content, levels off at 60-80% UDMH (by volume) and then falls off sharply. We reasoned that this phenomenon could be linked to the basicity

[1] The identity of NDMA was confirmed by comparison with authentic material (HPLC, GC, TLC, UV).

Table 3

Dependence of NDMA formation on initial [UDMH]/water ratio

% UDMH[1]	[NDMA] x 10^2 M[2]
100	0.728
90	5.36
80	10.3
70	7.53
60	12.4
50	7.18
40	4.90
30	2.09
20	0.807
10	0.256

[1] Volume per cent. UDMH in water.

[2] Average of 6-10 determinations.

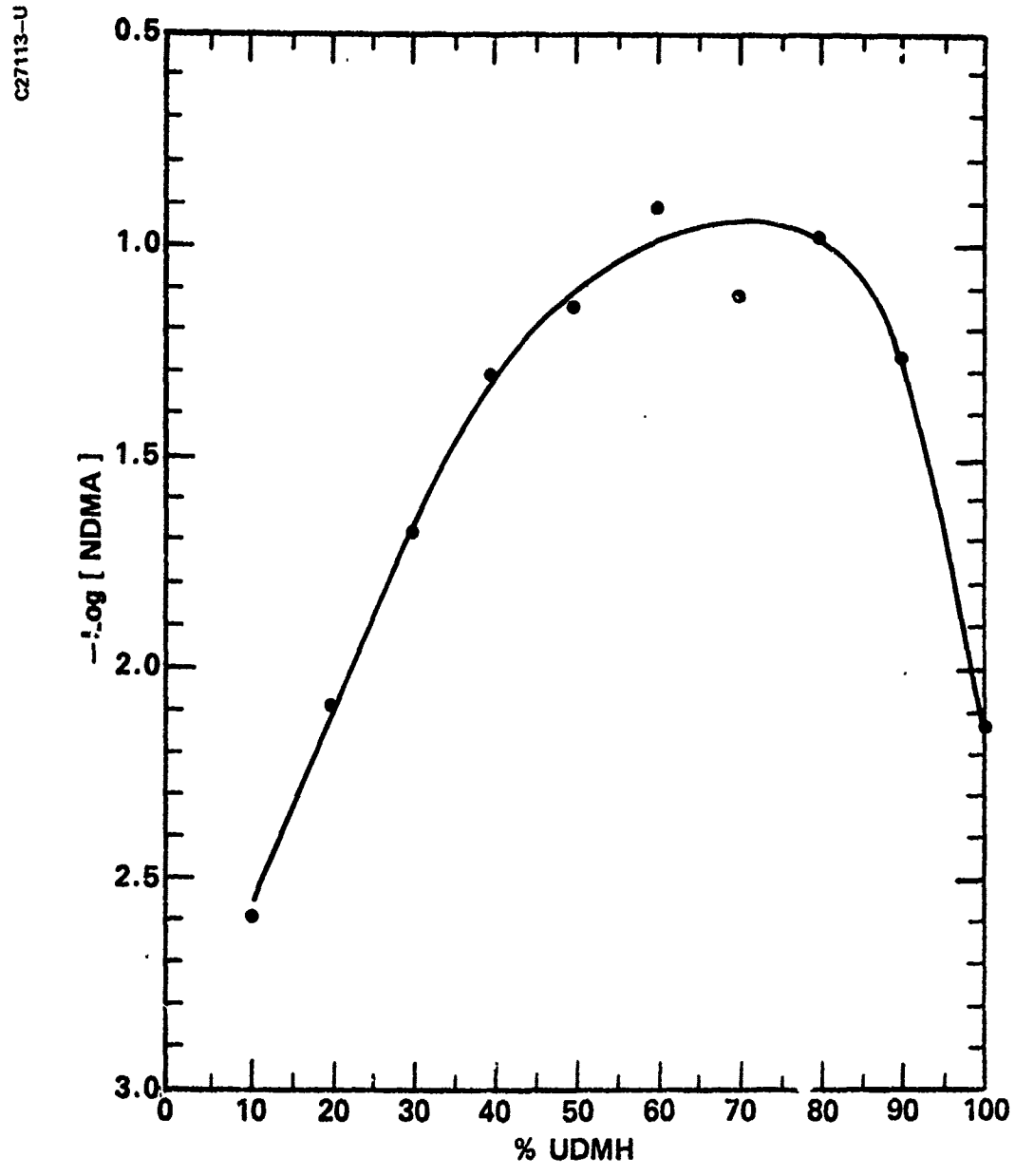
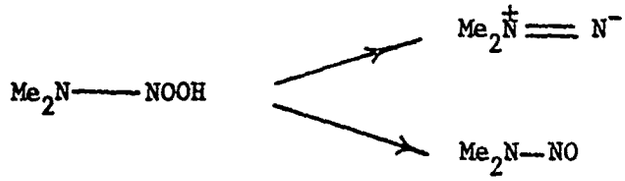


Fig. 3. Dependence of the extent of nitrosamine formation with the initial UDMH/water content.

of the medium, since the basicity increases with increasing UDMH content. In order to verify this hypothesis, experiments were performed where 10% solutions of UDMH in aqueous KOH were allowed to equilibriate with air. The experimental setup was modified somewhat for these studies, in that 200 µl solutions of 10% UDMH in water and in KOH were sampled periodically and analyzed for NDMA. These results are presented in Table 4, and indicate that NDMA is formed to a greater extent in the more alkaline medium. In a second series of experiments, the dependence of NDMA formation on the concentration of KOH in the solution was investigated. Solutions (200 µl.) of 10% UDMH in aqueous KOH solutions of varying strength were sealed in vials and analyzed for NDMA after a week. These data are presented in Table 5, and show that small amounts of alkali greatly enhance the final NDMA concentration.

These observations can, for the most part, be accounted for if the initial oxidation product of UDMH is a hydroperoxide, which then partitions into dimethyl-diazene and NDMA as shown below. If the conversion to NDMA occurs only under strongly alkaline conditions, then under neutral or



mildly basic conditions the hydroperoxide forms the diazene which subsequently dimerizes, rearranges, etc. to the tetrazene and other products. As the basicity of the medium is increased, however, progressively greater proportions of the diazene converts to NDMA, and thus nitrosamine formation occurs to a larger extent in UDMH/water mixtures. The decline in NDMA formation with even higher UDMH concentrations is not understood at this time. Possibly,

Table 4

Effect of base on the formation of NDMA

Day	NDMA (ppm)[1]	
	KOH soln.[2]	Aqueous soln.[3]
0	5	5
1	251	68
3	290	72
6	334	138
10	373	171
13	399	168
20	399	152

[1] Average of 2 determinations.

[2] 10% UDMH and 90% 0.1N KOH (by volume).

[3] 10% UDMH in water (by volume).

Table 5

Variation of final NDMA concentration with base strength

KOH(N)[1]	NDMA (ppm)[2]
0	288
0.16	543
0.40	639
0.81	685
1.60	695

[1] 10% UDMH + 90% of aq. KOH of the specified normality (by volume). The normality refers to the solution strength before the addition of UDMH.

[2] Average of 4 determinations.

since these solutions are far from aqueous, medium effects such as dielectric constants, differences in solvating ability, etc. predominate and lead to the observed fall off in the final NDMA concentration.

These findings are of direct environmental importance since they clearly show that NDMA is unlikely to be formed under environmental dilutions. Furthermore, they indicate that wet UDMH is more prone to be oxidized to NDMA than the dry compound, and every care to keep this material free from water should be taken.

(ii) Low UDMH Concentrations

In our earlier report we noted that the oxidation of UDMH in dilute aqueous solutions led to the formation of a product with an absorption maximum of 356 nm in acidic solutions. In order to quantify the conversion of UDMH to this product, a 4800 ppm solution of UDMH-HCl spiked with ^{14}C labeled material was brought to pH13 and kept under oxygen in a gas buret for three days. The resulting solution was analyzed by HPLC at two different wavelengths, and fractions corresponding to the observed peaks were collected and counted for radioactivity. The HPLC traces are illustrated in Figure 3, and product quantification is outlined in Table 6. The solution was also derivatized with salicylaldehyde (4) and analyzed for salicylaldehyde-dimethyl hydrazone. Only 6% of the total radioactivity was recovered from the hydrazone fraction, indicating that 94% of the UDMH had undergone oxidation.

The above results indicate that 20% of the radioactive material is converted to the compound absorbing at 356 nm. This does not, of course, imply that the molar conversion is 20%, since cleavage of the methyl nitrogen bond could occur. In any event, the results clearly establish that the compound in question is a major product of UDMH oxidation.

Identification of the product was pursued by allowing a solution of UDMH containing a trace amount of copper sulfate to react with oxygen in a gas buret for two days. The resulting solution was repeatedly extracted with ethyl-acetate and HPLC analysis of the dried concentrated extracts showed a predominance of the material absorbing at 356 nm. The concentrate was further analyzed by GC/MS (electron impact and chemical ionization) and the resultant spectrum is recorded in Figure 4. Not surprisingly, the base peak (m/e 28) in Figure 4 corresponds to nitrogen, and the two peaks observed at m/e 71 and 86 suggest fragmentation of a methyl group. Consequently, the compound appears to contain a nitrogen-nitrogen linkage as well as a methyl group.

Finally, since formaldehyde was observed to be an oxidation product of MMH, an experiment was initiated to test for the presence of this compound in dilute UDMH solutions. As before, solutions of UDMH were stirred for several hours in the presence of air, and samples were periodically withdrawn and analyzed for formaldehyde by the chromotrophic acid method (5). The results, listed in Table 7, clearly demonstrate that formaldehyde is a major oxidation product of UDMH.

4. Microbial Degradation of UDMH

In our earlier report we observed that degradation of UDMH in filter sterilized lake water gave rise to a compound with an absorption maximum at 326 nm which moved reversibly to 356 nm upon acidification. By contrast, degradation of UDMH in unsterilized water did not lead to products with significant absorption beyond 230 nm. We have now extended these initial results by monitoring the degradation of UDMH in waters collected in the Syracuse area.

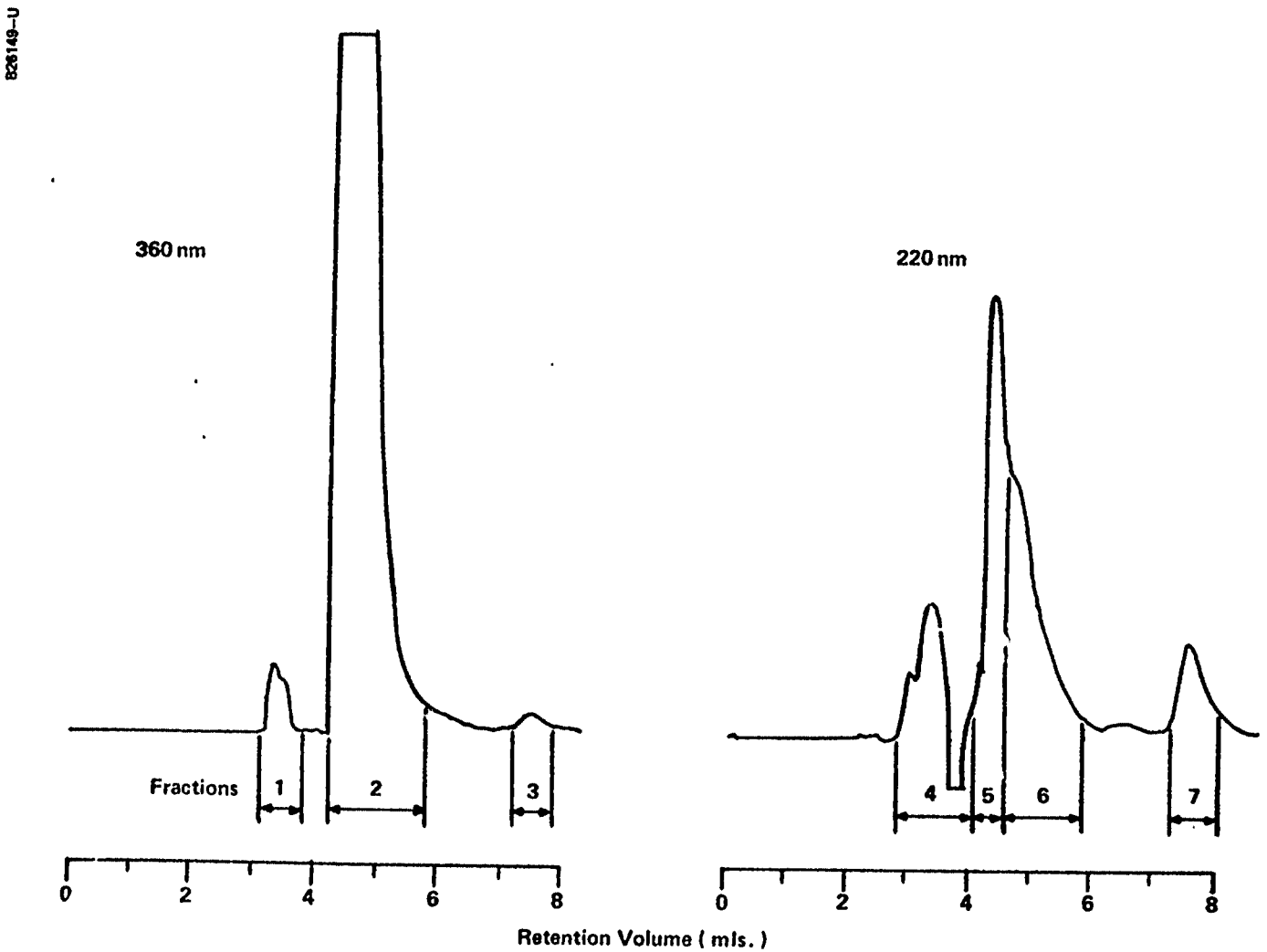


Fig. 3. HPLC analysis of the products of oxidation of UDMH.

Table 6

Quantification of products from the oxidation of ^{14}C UDMH

Fraction #	Anal. wavelength	% of total radioactivity
1	355 nm.	30
2	"	20
3	"	1
4	230 nm.	70
5	"	15
6	"	10
7	"	2

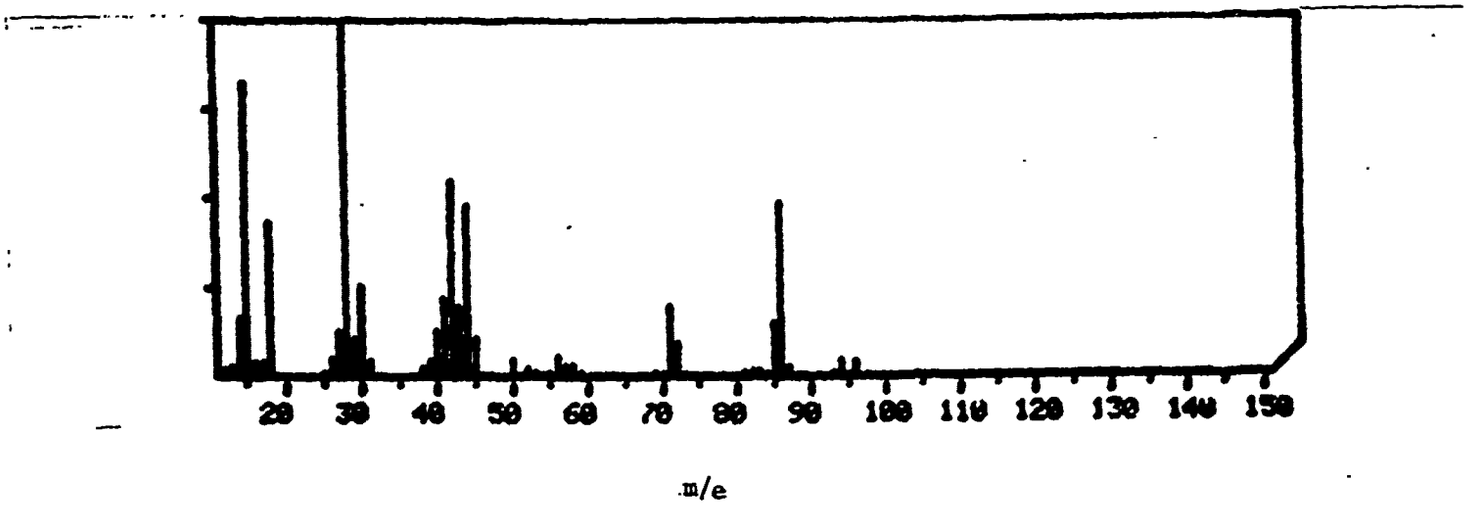


Fig. 4. Mass Spectrum of UDMH Oxidation Product

Table 7

Formation of formaldehyde from the oxidation of UDMH

[UDMH.HCl] x 10^4M	[Cu^{++}[x 10^5M	Minutes	% Conversion to H_2CO
1.10	1.00	6	20
"	"	13	9
"	"	19	15
"	"	29	19
"	"	37	23
"	"	60	29
5.20	1.00	3	5
"	"	6	9.8
"	"	10	12
"	"	19	16
"	"	25	19
"	"	42	27
"	"	58	27
"	"	16 hrs.	37
"	"	18 hrs.	44
5.70	0	16 hrs.	25

Several water samples were collected from each location. The samples corresponding to each location were composited, thoroughly mixed and the samples brought to our laboratories. Prior to undertaking the biodegradation studies, the microbial population (number of cells/ml) in the samples was determined using the standard serial dilution and plating techniques. A portion of water collected from each location was filter-sterilized by passing it through a 0.22μ Millipore filter.

A 100-ml sample of sterile or non-sterile water was added to a 250-ml Erlenmeyer flask and UDMH was added to the water at a concentration of 20 ppm. Each treatment included two replications. The water samples were incubated in the dark at 22°C. Aliquots of water were removed at appropriate intervals over a period of two weeks and analyzed by UV spectrophotometry and HPLC. The microbial population in the samples was also determined two weeks after adding UDMH.

The initial and final microbial population (cells/ml water) in the water samples used in our studies is shown below.

Jamesville Reservoir ($7 \times 10^4 - 3 \times 10^5$)

Seneca River ($3 \times 10^3 - 3 \times 10^5$)

Oneida Lake ($1 \times 10^5 - 9 \times 10^5$)

Our results show that the degradation of UDMH proceeds at essentially the same rate in sterile and non-sterile samples taken from various locations. The degradation of UDMH in all water samples resulted in a compound(s) absorbing at 326 nm. A similarity in the rates and products of degradation of UDMH in sterile and non-sterile water samples suggests that microbial degradation is not an important process in determining the environmental fate of UDMH.

5. Bioconcentration of UDMH in Aquatic Organisms

(i) Fish

Based upon our studies on the chemical properties of UDMH in solution, several limitations were placed on the design of experiments which would define the bioconcentration potential of UDMH in aquatic organisms. Because of the rapid, oxidative decomposition of UDMH, forming a number of products of diverse chemical nature, it was impossible to measure uptake of UDMH alone. In theory, rapid continuous flow systems are preferred for such labile chemicals, since the accumulation of transformation products is prevented. However, concentrated stock solutions are required for such apparatus and we have found it impossible to prepare and maintain oxygen-free stock solutions of UDMH.

Concentrated stock solutions would also degrade to different products than dilute, and more environmentally relevant, solutions. Other alternatives, such as using reduced oxygen tension or low pH (≤ 5) to retard

UDMH breakdown, were also discarded as they might also give potentially misleading results (i.e. different products) in terms of environmental conditions. We therefore planned our experiments within the constraints of static and static-renewal systems. Benefits of this approach included reduction in the amount of ^{14}C required (low uptake and high toxicity of UDMH dictated the use of low concentrations of UDMH at high specific activity), enabled experimental set-ups to be confined to fume-hoods (minimizing worker exposure to known and potential carcinogens) and reduced problems in disposing of highly radioactive and carcinogen-containing wastewater.

Our general experimental approach was to examine uptake of UDMH and/or its degradation products under several conditions in order to determine which situations may lead to a high level of bioconcentration in the environment. Lack of sensitive analytical methodologies prevented identification of UDMH or products formed in water and accumulated in fish. However, by measuring the uptake of ^{14}C from the original ^{14}C-methyl UDMH, some judgement of "worst possible situations" (i.e. that all of the bioconcentrated residue constitutes a toxic or carcinogenic material) can be made.

Previous work with UDMH established that the toxicity of UDMH solutions to fish was highly dependent on the age of the solutions at the time of exposure. Older solutions were less toxic than freshly prepared solutions.(7)

To determine what, it any, effect age of UDMH solution had on bioconcentration of UDMH and/or products, two replicate solutions of 1 ppm ^{14}C-UDMH·HCl were prepared. Six bluegills were immediately placed into one replicate (loading 2 g fish/liter) and the other replicate remained without fish. Both solutions were mildly aerated (ca. 50 bubbles/min.). The ^{14}C

content of both solutions was measured initially and at daily intervals thereafter. Two fish were sacrificed at 24, 48, and 72 hours for analysis of whole fish ^{14}C-residues. After 72 hours, six fish were added to the previously empty (no fish) and now 72 hr. old UDMH solution, and uptake of ^{14}C measured at 24, 48, and 72 hours of exposure. The results are given in Table 8. Uptake equilibria were rapidly reached under both conditions and bioconcentration was approximately twice as high in the intially fresh solution as opposed to the aged solution. However, uptake is low in both cases and the difference in results may be exaggerated by the combination of low uptake and small sample sizes. The loss of ^{14}C from the solutions was linear with time, with a half-life of approximately 192 hours in both cases. Since the half-life of disappearance of UDMH in natural water is of the order of 10 hours, the volatilized ^{14}C probably represents transformation products. The volatile material may be relatively hydrophobic (ionized species would presumably remain in water) and more likely to accumulate in fish than more polar materials. The loss of ^{14}C over the three day aging period could therefore have considerably lowered the level of "bioconcentratable" material prior to introduction of fish, leading to the observed difference in uptake between the two situations.

A further study was conducted to determine the uptake of ^{14}C under conditions where ^{14}C-UDMH was more prevalent in the exposure medium. Therefore, twenty-four bluegills were placed in 10 liters of 0.1 ppm ^{14}C-UDMH·HCl. The fish were transferred to new exposure solution daily. Three fish were sampled at intervals of 24, 48, 72, and 148 hours and analyzed individually for ^{14}C content. After 148 hr. exposure, the remaining fish were

Table 8. Effect of Solution Age on the Uptake of UDMH and/or Products in Fish

Sample Time	Bioconcentration Factor	
	Non-aged	Aged
24	1.1	1.3
48	3.4	2.0
72	3.7	1.7

Values are the results from two whole fish pooled for analysis at each time interval, and are expressed as bioconcentration factor due to changing levels of ^{14}C in the water.

placed in uncontaminated, flowing water and the ^{14}C remaining in the fish after initiation of depuration was measured at 24, 72, 144, and 216 hours.

The data presented in Table 9 indicate that an equilibrium is achieved between ^{14}CUDMH/products in fish and in water within 72 hours of initial exposure. A bioconcentration factor [chemical] fish/[chemical] water of 0.91 was obtained at equilibrium for whole fish and of 0.75 for the edible portion of the fish. Also, the accumulated material is rapidly eliminated from the fish upon transfer to noncontaminated water. Over one-half of the ^{14}C was eliminated in the first day of depuration, and less than 10% remained after 9 days of depuration.

Therefore, under these experimental conditions, UDMH and/or its transformation products do not accumulate in fish to levels greater than found in the water, and the material which does enter the fish is rapidly eliminated when the source of contamination is removed. The level of uptake observed (bioaccumulation factor = 0.9) is somewhat lower than that seen when the UDMH solution was not renewed (bioaccumulation factor = 3.7). The present study was done at 0.1 ppm UDMH in water while the previous study was done at 1.0 ppm (initially). The nature and quantity of products formed could be influenced by this difference. In addition, renewing exposure solutions daily decreases the effective concentration of products which may be taken up, providing a period of time when such products are absent from the water. Assuming that it is the products of UDMH (rather than UDMH itself) which are predominately accumulated, the fresh solutions could provide a depuration medium for some time. The data in Table 9 indicates that depuration of ^{14}C accumulated under the renewal conditions is very rapid.

Table 9. Uptake and Elimination of ^{14}C-UDMH in Bluegills (solutions renewed daily)

	ppm ^{14}C-UDMH·HCℓ equivalent[1]		
Exposure Phase (hours)	Edible	Non-Edible	Whole Fish
24	0.0442	0.0610	0.0517
48	0.054	0.0856	0.0673
72	0.1031	0.1226	0.1031
168	0.0810	0.1329	0.1031
Depuration Phase (hours)[2]			
24	0.0358 (44.2)[3]	0.0598 (50.0)	0.0456 (44.8)
72	0.0219 (27.0)	0.0393 (29.6)	0.0294 (28.9)
144	0.0120 (14.8)	0.0362 (27.2)	0.0225 (22.1)
216	0.0092 (11.4)	0.0100 (7.5)	0.0096 (9.4)

[1]Values are the average from 3 fish analyzed individually at each sampling period.

[2]Depuration was initiated after 168 hours of exposure.

[3]Values in parentheses are the percent of original ^{14}C-residues remaining after initiating depuration.

Our chemical studies have shown that the nature of UDMH transformation products is dependent on the initial UDMH concentration present. In view of our results showing a very low degree of bioconcentration from UDMH concentrations of 1 ppm or less, we conducted a study to determine bioconcentration of UDMH/products which may form under more concentrated conditions. ^{14}C-UDMH was dissolved in lake water to give a 1.3% solution, sealed under air, and held for two days at 21°C. At this point, the solution was analyzed by HPLC for the presence of N-nitrosodimethylamine and tetramethyltetrazene. No tetrazene was detected and the nitrosamine content was determined to be 0.3% of the ^{14}C-chemical present by radiometric assay. Aliquots of this solution were taken and diluted with water to give a concentration of 0.066 ppm ^{14}C-UDMH equivalent. The low concentration was chosen to avoid possible toxic effects from the nitrosamine. Bluegills were placed in the solutions at loading ratio of 5g/ℓ and were sacrificed for analysis at 3 and 6 days of exposure. The concentration of ^{14}C-material at these times was 0.03 ppm (^{14}C-UDMH equivalent)(average of three fish per sample), indicating that equilibrium is rapidly achieved. The bioconcentration factor of ^{14}C was 0.41. Therefore, products of UDMH formed under rather concentrated conditions, such as from a UDMH spill, are not accumulated by fish. This assumes that one component of the ^{14}C-labelled material, comprising a small fraction of the total, is not concentrated to the exclusion of the remainder of the mixture. If such were to occur, the bioconcentration factor of the component could be considerable. However, the minor nature of the component would prevent the subsequent residues from achieving hazardous levels.

One observation of note is that through the 6 day exposure period, the ^{14}C measured in the water declined by 13.5%. In contrast, ^{14}C

levels in the low concentration study reported above decreased by 34.3% in the same time span. This may be additional evidence that the proportion and nature of UDMH products formed is concentration dependent.

(ii) Daphnia

As an additional indicator of the bioconcentration potential of UDMH and its degradation products, uptake studies were conducted with the cladoceran, Daphnia magna.

As with fish, two approaches were taken. Uptake was studied with the concentrated UDMH solution aged for two days before dilution, and a fresh UDMH solution.

Mixed age populations of Daphnia were placed in solutions of an artificial daphnia medium containing 0.35 ppm of the aged ^{14}C-UDMH and products or 0.14 ppm fresh ^{14}C-UDMH. At intervals of 24, 48, and 72 hours, the daphnia were collected by filtration on pre-weighed millipore filters, air-dried, and weighed. They were then homogenized in scintillation cocktail for measurement of ^{14}C.

The results are given in Table 10. As with the fish, uptake appears to reach equilibrium within 72 hours for both aged and fresh solutions. Uptake is somewhat higher for the aged solution (bioconcentration factor of 26 vs. 19 for the fresh solution). The degree of uptake observed is higher for daphnia than for fish. This might relate to less efficient excretory mechanisms or different biochemical composition leading to greater uptake. Also, adsorption to or reaction with the exoskeleton may result in a higher uptake. To check the latter possibility, a depuration study will be conducted.

In summary, although uptake is considerably higher for Daphnia than in fish, the magnitude is not such as to constitute an environmental hazard.

Table 10. Uptake of ^{14}C-UDMH and Products in *Daphnia* Uptake ppm ^{14}C-UDMH equivalent[1]

Time Sample (hours)	Type of UDMH Solution	
	Fresh[1]	Aged[2]
24	1.35[3]	4.77
48	1.22	10.05
72	2.62	9.35

[1]Exposure water prepared by dissolving ^{14}C-UDMH in water for immediate use.

[2]Exposure water prepared from a 1.3% UDMH·HCℓ solution aged for 2 days before experiment.

[3]Values are the average of duplicate samplings at each time.

6. Bioconcentration of Tetrazene in Fish

A preliminary study was performed in order to judge the bioconcentration potential of tetramethyl tetrazene, a potentially important oxidation product of UDMH. Tetrazene may have a higher probability of accumulating in fish than UDMH or other degradation products due to its relatively non-polar structure. ^{14}C-Methyl tetrazene was prepared by bromine oxidation of ^{14}C-UDMH, and its identity confirmed by TLC, HPLC, and U.V. analysis. Two solutions of ^{14}C-tetrazenes were prepared and 4 bluegills were placed in one while the other was maintained as a non-fish control. Both solutions were slowly aerated. After 5 hours, we found that 19% (control) to 28% (fish) of the original ^{14}C was not detected in the water and presumably was lost through volatilization. After 72 hr, only 6% (fish) to 12% (control) of the ^{14}C remained. The bioconcentration factor in the fish at these times, based on ^{14}C remaining in the water, was 1.4 at 5 hours and 9.5 at 72 hours.

Because the 10-fold bioconcentration may not reflect an equilibrium value, these results justify further investigation of tetrazene bioconcentration, using a flow-through system to maintain a constant concentration in the exposure water. Because of a lack of a sufficient amount of ^{14}C-methyl tetrazene needed for flow-through studies, we were not able to pursue our studies on the bioaccumulation of this chemical.

C. Summary

In the present phase of the study we have determined the kinetics of oxidation of MMH in aqueous solutions as a function of pH and catalyst concentration. It is evident from our data that in non-acidic waters MMH will not persist for longer than one or two days. A complete mass balance of MMH has not been obtained; however, one of the major oxidation products is formaldehyde.

We have addressed the important question of NDMA formation from UDMH in UDMH/water mixtures. Our results clearly illustrate that under conditions of limiting oxygen, the extent of nitrosamine formation is a function of the water content of the solution, with optimum conversion occurring in 50-80% of UDMH (by volume) in water. Significantly, the formation of nitrosamine rapidly decreases with increasing dilution of the UDMH solution, which indicates that oxidation of UDMH to NDMA is unlikely to occur under environmental conditions. The products of UDMH in dilute solutions have not been completely characterized. However, formaldehyde has been identified as a major product. A number of studies have also been conducted to determine the role of naturally occurring microbial populations on the degradation of UDMH in water. Spectrophotometric analysis revealed no differences between the test samples and their sterile controls.

The following conclusions can be made regarding the uptake of UDMH by aquatic organisms.

1. Bioconcentration factor of UDMH/products in fish obtained from an initial 1 ppm UDMH solution, not replaced over a 3 day exposure period, was 3.7.
2. Bioconcentration factor of UDMH/products in fish obtained when an initial

1 ppm solution was allowed to stand 3 days before addition of fish, and not replaced over a 3 day exposure period was 1.8.

3. Bioconcentration factor of UDMH/products in fish obtained from 0.1 ppm UDMH solution replenished daily was 0.91.
4. Bioconcentration factor of UDMH/products in fish obtained from a two day-old 1.3% solution of UDMH in water, subsequently diluted, was 0.41.
5. Bioconcentration of UDMH/products in Daphnia is higher than in fish (factors of 19 to 26) but is not likely to constitute an environmental hazard.

These results show that the potential for bioaccumulation of UDMH and products of transformation in fish and Daphnia is quite low under a variety of conditions. This is not surprising since UDMH and its transformation products are small, relatively polar molecules, and the excretory mechanisms of fish are particularly well suited for the elimination of a variety of similar nitrogenous materials. This is borne out by the rapid depuration rates observed in our studies.

D. Experimental Procedures

UV spectra were recorded on a Cary 14 spectrophotometer and HPLC analyses were performed on a Waters M6000A instrument with μC 18 Bondapack as the stationary phase and 99% (1% acetic acid in water) - 1% acetonitrile as the mobile phase. GC measurements were conducted on a Hewlett Packard 5730A FID chromatograph, and the oxidation kinetics were obtained from a YSI model 53 instrument.

UDMH, MMH and NDMA were obtained from the Aldrich Chemical Co. and tetra-methyl-tetrazene was synthesized according to McBride and Kruse (6). ^{14}C labeled UDMH·HCℓ 98% radiochemical purity, 2 mCi/mMole was obtained from California Bionuclear Corp.

Isolation of the Products of Oxidation of UDMH

The experiments relating to the formation of NDMA from concentrated solutions of UDMH were conducted as follows. Tubes of capacity 25 ml were flame dried, sealed tightly after introduction of the reagents with aluminum foil screwcaps, after which several layers of parafilm were wrapped around the seal. Volumetric flasks were used in the initial experiments but were discarded in favor of screw cap tubes since leakage of the solution in the former was observed to occur. Analyses of the samples were carried out by HPLC, and the results quoted in Tables 3 and 7 are averages of 2-6 determinations. In general, better than 5% reproducibility was routinely obtained when samples were analyzed from the same tube, but the variance increased considerably when samples from two identical tubes were analyzed. This could possibly arise from the residual water adsorbed onto the glass surface, since the concentration of water in solution effectively controls the extent of nitrosamine formation.

Isolation of the oxidation product referred to in the text (Sec. B.3) was achieved by collecting factions of the material by preparative HPLC. The pooled factions were extracted four times with equal volumes of ethyl acetate, and the dried concentrated ethyl acetate extracts were analyzed by HPLC using methanol as the mobile phase. Two peaks were observed, and the compound corresponding to the major peak was collected by preparative HPLC, concentrated and analyzed by GC-MS. An interpretable mass spectrum was not obtained.

Formaldehyde was analyzed by the chromotropic acid method (5). Reference formaldehyde solutions were analyzed by iodometric oxidation.

Bioconcentration of UDMH in Fish

Bluegill sunfish (Lepomis macrochirus Raf.), 1"-2" in length were obtained from the National Fish Hatchery, Orangeburg, S.C. governmental fish hatchery and acclimated to laboratory conditions before being exposed to UDMH.

^{14}C labeled UDMH·HCℓ salt 98% radiochemical purity, 2 mCi/mMole was obtained from California Bionuclear Corp., Sun Valley, CA.

Radiometric analysis of ^{14}C uptake in fish was measured in the following manner. At appropriate intervals, the exposed fish were removed from solution, briefly rinsed in clean water, and frozen. After freezing, the fish were separated into edible (muscle, skin) and non-edible (head, internal organs) portions, weighed, and blended twice in five volumes of methanol. The methanol extracts were pooled for radiometric determination by liquid scintillation counting using Instagel scintillation cocktail. The ^{14}C present in the extracted residue was measured by solubilization in NCS tissue solubilizer,

followed by scintillation counting. Values for uptake were calculated and expressed as ppm UDMH·HCℓ equivalent given the total ^{14}C detected and the specific activity of the starting material.

The water used to prepare the exposure solutions was obtained by dechlorination of municipal drinking water by activated carbon filtration.

The pH of the solutions of UDMH and products at the beginning of exposure was 7.3 - 7.7.

E. Literature Cited

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F. Publications Resulting From the Research

The following manuscript has recently been submitted for publication in the Journal of Environmental Science and Technology:

"Kinetics of Aqueous Oxidation of Methylhydrazines: Factors Controlling the Formation of 1,1-Dimethylnitrosoamine", Sujit Banerjee, Harish C. Sikka, Richard Gray, Christine Kelly and Edward Pack.

G. Professional Personnel Associated with the Research Effort

Harish C. Sikka, Ph.D.
Sujit Banerjee, Ph.D.
Henry T. Appleton, Ph.D.

H. Interactions (Coupling Activities)

A paper entitled "Environmental Fate of 1,1-Dimethylhydrazine" was presented by Harish C. Sikka at a symposium on the "Review of Air Force Sponsored Basic Research in Environmental Protection and Toxic Hazards" in Los Angeles, CA, January 1979.